Original Article

The Effects of Amiloride and Age on Oxygen Consumption Coupled to Electrogenic Sodium Transport in the Human Sigmoid Colon

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ABSTRACT

Background/Aim: Aerobic metabolism is necessary for ion transport in many transporting epithelia, including the human colonic epithelium. We assessed the effects of the epithelial sodium channel blocker, amiloride, on oxygen consumption and short-circuit current of the human sigmoid epithelium to determine whether these effects were influenced by the age of the subject. **Materials and Methods:** Segments of the sigmoid colon were obtained from the safety margin of resections performed in patients of 62–77 years of age. Isolated mucosa preparations were obtained and mounted in airtight Ussing chambers, fit for simultaneous measurement of short-circuit current and oxygen concentration, before and after blocking epithelial sodium channels with amiloride (0.1 mmol/L). Regression analyses were performed to assess the associations between short-circuit current, oxygen consumption, and age of the subject as well as to define the relationship between the decreases in short-circuit current and oxygen consumption after blockade. **Results:** Epithelial sodium channel blockade caused an 80% reduction in short-circuit current and a 26% reduction in oxygen consumption. Regression analysis indicated that both changes were significantly related (r = 0.884; P = 0.0007). Oxygen consumption decreased by 1 μmol/h/cm^2 for each 25 μA/cm^2 decrease in short-circuit current. Neither short-circuit current nor oxygen consumption had any significant relationship with the age of the subjects. **Conclusion:** The decrease in epithelial oxygen consumption caused by amiloride is proportional to the decrease in short-circuit current and independent of the age of the subject.

Key Words: Aerobic metabolism, amiloride, ENaC, Ussing chamber

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An important function of the colon is absorption of sodium and water.[1] In the human descending colon, a large fraction of electrogenic transepithelial transport is due to sodium reabsorption. This process takes place by a mechanism, which involves a transcellular pathway dependent on apical epithelial sodium channels (ENaC) and the basolateral Na, K-ATPase.[2-4] This absorptive pathway is a target for aldosterone action.[1,5] Aldosterone increases electrogenic sodium absorption, decreases colonic crypt permeability,[6] and prevents back-leakage.[7]

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Besides contributing to conservation of sodium chloride and water, the normal function of electrogenic sodium absorption in the distal colon is important for the proper dehydration of feces. The derangement of electrogenic sodium absorption is a major cause of nonsecretory diarrhea in inflammatory bowel disease.[8] Impaired electrogenic sodium absorption has been demonstrated both in ulcerative colitis[9] and in microscopic colitis,[10] probably caused by inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β.[11,12] Anti-inflammatory treatment is associated with an improvement in electrogenic sodium absorption.[10,13]

Electrogenic ion transport is dependent on oxidative energy metabolism[14] and demands a continuous oxygen supply.[15] Despite the well-known oxygen dependence of epithelial ion transport, there are relatively few papers published on colonic epithelium oxygen consumption under conditions preserving vectorial ion transport, and these have studied

nonhuman species, for example, rabbits[16] and rats.[17] We have recently reported data for the human colon, indicating that a significant fraction of epithelial oxygen consumption is associated with electrogenic sodium transport.[18]

In the present report, we characterize the relationship between amiloride-sensitive electrogenic sodium transport and epithelial oxygen consumption in the human colon. Additionally, we assessed whether the age of the subject influences the effect of amiloride on short-circuit current or oxygen consumption.

MATERIALS AND METHODS

Recruitment of volunteers

We carried out this study in compliance with the 6th Revision (2008) of the Declaration of Helsinki. The protocol was approved by the Committee on Bioethics of our Faculty of Medical Sciences. We secured written informed consent from patients who were scheduled to undergo extirpation of left colon adenocarcinoma. We obtained samples from 10 patients (4 female) with an age range of 62–77 years.

Preparation of mucosal samples

We took a 3-cm long segment (ring) of the sigmoid colon from each patient. Each selected segment was considered free of disease upon visual inspection by the operating surgeon. We rinsed the segments free of debris and placed them in an oxygen-saturated solution, kept at 4°C, and immediately carried to the laboratory. We dissected each segment to obtain an isolated mucosa preparation as previously described.[18] We cut and immediately fixed a portion of the preparation for standard light microscopy to confirm the extent of dissection and the lack of signs of disease. Then, we mounted the remaining tissue as a flat sheet in a modified Ussing chamber.

Ussing chamber

We used a modified Ussing chamber, which could be hermetically closed to allow continuous monitoring of oxygen concentration through polarimetric oxygen probes, as previously described.[17,18] The chamber has a window of 1 cm^2. Each hemichamber has a small magnetic bar in its bottom for continuous mixing of contents when placed on a magnetic stirrer (HI 300N, Hannah Instruments, Woonsocket, Rhode Island, USA). Each hemichamber has a bubble trap through which drugs may be injected, and a port for inserting a polarimetric oxygen probe (CellO × 325) connected to WTW Oxi 340 oxygen meter (WTW GmbH, Oberbayern, Germany). The probes allow continuous measurement of oxygen concentration and temperature. Knowing the volume of the chamber, we calculated the oxygen consumption rate from the change in oxygen concentration, taking into account the solubility of oxygen in solution at 37°C.

Solution and amiloride

We filled the chamber with a solution containing 145 mM NaCl, 1.6 mM K_2HPO_4, Units of concentration (mM) are lacking for KH_2PO_4, $MgCl_2$, and $CaCl_2$ and 5 mM d-glucose (pH 7.40). We gassed the solution with 100% oxygen to saturation. For each experiment, we freshly dissolved amiloride (Sigma-Aldrich, Saint Louis, Missouri, USA) in dimethyl sulfoxide to yield a final concentration in the mucosal side of the chamber of 0.1 mmol/L.

Mucosal electrophysiology

Calomel electrodes were connected to each hemichamber through 3% agar-in Ringer bridges to record transepithelial potential difference. An amplifier, with correction for bridge asymmetry and solution resistivity, allowed passing current through $Ag/AgCl_2$ electrodes for clamping the transepithelial potential difference at 0 mV. Transepithelial resistivity (specific resistance) was calculated according to Ohm's law.

Experimental protocol

We performed the experiments at 37°C, continuously recording short-circuit current, except for periodic releases to measure open-circuit transepithelial potential difference.

When the short-circuit current reached a plateau, we measured baseline oxygen consumption during a 30-min period. We then added amiloride. After the short-circuit current was again stable, we measured oxygen consumption for a second 30-min period.

Statistical analysis

We performed the statistical analysis with Prism for Windows, version 5.04 (Graph Pad, San Diego, CA, USA). We evaluated changes in electrophysiological variables and oxygen consumption with a two-sided Student's *t* test for paired samples, after checking that the data did not significantly deviate from a Gaussian distribution with D'Agostino and Pearson omnibus normality test. We assessed the relationship between changes in oxygen consumption and short-circuit current caused by amiloride by linear regression analysis with a check for significant deviation from linearity. We also performed a regression analysis to check whether the age of the subjects had any influence on short-circuit current or oxygen consumption. We chose a significance level of 0.05 and report the data as mean ± standard error of mean.

RESULTS

None of the samples studied showed signs of neoplastic disease or inflammation by light microscopy. Figure 1 shows

a typical result, namely, a healthy epithelial sheet virtually free of submucosal tissue.

In Table 1, we show values of short-circuit current, transepithelial potential difference, transepithelial resistivity, and oxygen consumption rate at baseline and after amiloride addition. Amiloride caused a 48.2 ± 4.7 μA/cm^2 decrease in short-circuit current and a 1.86 ± 0.17 μmol/h/cm^2 decrease in oxygen consumption rate, representing reductions of approximately 80% and 26%, respectively. Transepithelial potential difference decreased by 75% and transepithelial resistivity increased by 16%. All of these changes were statistically significant ($P < 0.0001$).

We found no significant relationship with regression analysis between age of the subjects and baseline short-circuit current ($P = 0.860$), baseline oxygen consumption rate ($P = 0.234$), change in short-circuit current ($P = 0.981$), or change in oxygen consumption rate ($P = 0.947$) after addition of amiloride [Figure 2]. In no case did the relationship deviate significantly from linearity, nor was the slope significantly different from zero (all $P > 0.05$).

On the other hand, we found a significant linear correlation between the decrease in short-circuit current and the decrease in oxygen consumption rate after addition of amiloride, such that the latter decreased by 0.04 μmol/h/cm^2 for each μA/cm^2 of reduction in the former. The result of the regression analysis between oxygen consumption rate and short-circuit current is shown in Figure 3.

DISCUSSION

Baseline values of short-circuit current measured in this study are similar to those previously reported by us[18] and other authors.[19,20] Present data on baseline oxygen consumption rate are also in agreement with our previous work.[18] In that report, ouabain added to the serosal hemichamber caused a large decrease in short-circuit current and oxygen consumption rate, but the number of observations was too small for assessing correlation.[18]

Table 1: Electrophysiological variables and oxygen consumption in the isolated mucosa of human sigmoid colon

	Baseline	Amiloride
Short-circuit current (μA/cm^2)	58.5±4.9	10.0±1.1*
Transepithelial potential difference (mV)	6.67±0.55	1.29±0.13*
Transepithelial resistivity (Ω.cm^2)	115.0±4.2	131.4±4.1*
Oxygen consumption (micromol/h/cm^2)	7.02±0.18	5.16±0.22*

Amiloride was added to the mucosal hemichamber for a final concentration of 0.1 mmol/L. Values are mean±SEM. Data were analyzed with a two-sided Student's t test for paired samples. *All differences between columns are significant at $P<0.0001$

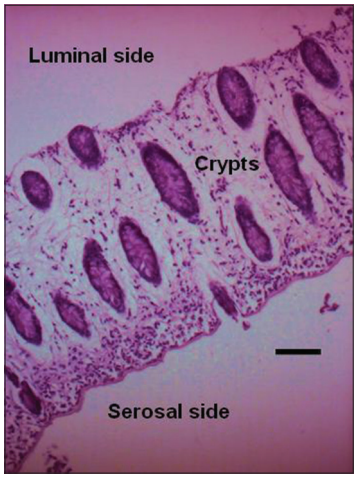

Figure 1: A sample of isolated sigmoid colonic mucosa from a 66-year-old male patient, showing extent of dissection and lack of signs of disease. Hematoxylin and eosin stain, Calibration bar = 100 μm

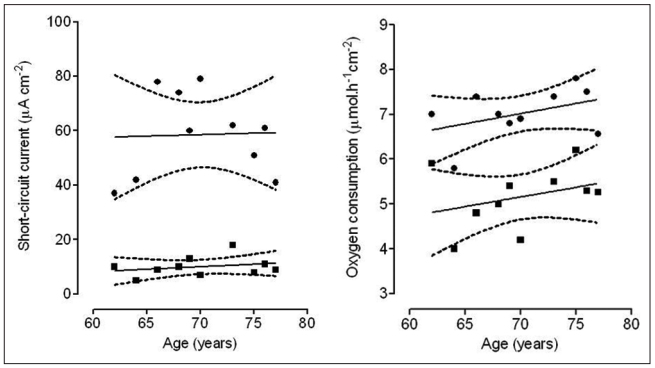

Figure 2: Regression analysis of short-circuit current (μA/cm^2, upper panel) and oxygen consumption rate (micromol/h/cm^2, lower panel) versus age under baseline conditions (circles) and after addition of amiloride (squares). The 95% confidence intervals are shown in trace lines. No regression line was significantly different from zero

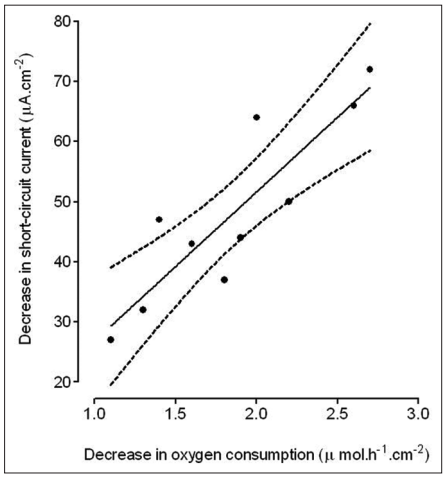

Figure 3: Linear regression analysis of the reduction in short-circuit current and the corresponding reduction in oxygen consumption rate in isolated mucosa preparations from human sigmoid colon ($n = 10$). $R = 0.884$; $P = 0.0007$

Similarly, the magnitude of the effect of amiloride on short-circuit current is within the range reported by other researchers.[2,3] We are not aware of reports on the effect of amiloride on epithelial oxygen consumption rate. Our present data show that amiloride causes a significant decrease in oxygen consumption rate, and that this decrease is highly correlated with the decrease in short-circuit current caused by the drug. This indicates that electrogenic sodium transport demands about one quarter of the total oxygen consumption of this epithelium. Therefore, this report corroborates the close relationship between sodium absorption and aerobic metabolism in the human sigmoid colon.

All isolated mucosa samples were obtained from the sigmoid colon of subjects older than 60 years. Because it is known that the sodium reabsorption capacity of the kidney declines with age,[21] there might be some concern regarding whether present results are significantly affected by the age of the subject. However, within the age range of our participants (62–77 years), no significant relationship was found between age and short-circuit current or oxygen consumption rate, or their decreases caused by amiloride. This finding agrees with the results of Greig *et al*. regarding electrogenic transport in human sigmoid colon.[22] They found no significant differences in subjects who were 20–40 years of age and in subjects older than 70 years in the expression of ENaC and Na, K-ATPase, baseline transepithelial potential difference, response to amiloride, or response to a synthetic mineralocorticoid. They concluded that, unlike what is observed in the distal nephron, electrogenic sodium absorption does not decline with age in the normal human distal colon.[22] Our own findings additionally suggest that the coupling between electrogenic transport and oxygen consumption is preserved even at advanced age.

From a clinical viewpoint, our results suggest that derangements in ion transport observed in inflammatory bowel diseases[8-10] might be, at least in part, due to reduced oxygen availability for ATP synthesis, for example, due to increased utilization of oxygen for the generation of reactive oxygen species. This hypothesis may be tested in epithelial samples from patients with colitis.

We should point out some limitations of our study. First, our results come from a convenience sample. Second, the size of the sample did not allow us to test whether there are gender differences in electrogenic transport and oxygen consumption, influence of age, or effect of amiloride. Third, the age range of the patients was limited to just 15 years.

CONCLUSION

The present report shows that amiloride proportionally lowers sodium absorption and oxygen consumption in the human distal colon under conditions which preserve vectorial ion transport. These effects of amiloride, which corroborate a tight coupling between electrogenic transport and oxygen consumption, is not influenced by the age of the subjects.

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